

Growth and Development of *Encarsia formosa* (Hymenoptera: Aphelinidae) in the Greenhouse Whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae): Effect of Host Age

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The tiny parasitoid wasp, *Encarsia formosa*, has been used successfully to control greenhouse whiteflies (GHWFs) in greenhouses in many countries throughout the world. Therefore, there has been considerable interest in developing methods for artificially rearing this wasp. However, little information is available concerning the regulation of its development including the host-parasitoid interactions that are required for the parasitoid to complete its life cycle. Here we confirm that parasitoid developmental rates differ significantly based upon the host instar parasitized. Development was faster when 3rd and 4th instar GHWFs were offered for parasitization than when 1st or 2nd instars were used. Our results show that it is primarily the embryo and the first two parasitoid instars that exhibit prolonged developmental times when 1st and 2nd instar whiteflies are parasitized. Although percent emergence was not affected by host age at the time of parasitization, adult longevity as well as adult emergence pattern varied greatly depending upon the instar parasitized. When 3rd and 4th instar GHWFs were selected for oviposition, adult wasps lived significantly longer than when 1st or 2nd instars were used; also, there was a sharp emergence peak on the 2nd day after emergence was first observed (reduced or absent when 1st or 2nd instar GHWFs were parasitized) and the emergence period was reduced from between 8 and 11 days to 5 days. In general, the younger the host instar parasitized, the less synchronous was parasitoid development. Previous reports that *E. formosa* will not molt to the 2nd instar until the host has reached its 4th instar were not confirmed. When 1st instar host nymphs were parasitized, 2nd instar parasitoids were detected in 3rd instar hosts. Importantly, however, no matter which instar was parasitized, the parasitoid never molted to its last instar until the host had reached Stage 5 of its last instar, a stage in which host pharate adult formation has been initiated. It appears, then, that a condition(s) associated with host pharate adult formation is required for the parasitoid's final larval molt. Results reported here should facilitate the development of in vitro rearing systems for *E. formosa*. Arch. Insect Biochem. Physiol. 49:125–136, 2002. Published 2002 Wiley-Liss, Inc.[†]

KEYWORDS: greenhouse whitefly; *Encarsia formosa*; parasitoid development; parasitoid morphology; host-parasite interactions

INTRODUCTION

Encarsia formosa parasitizes several whitefly species and is a valuable biological control agent for the greenhouse whitefly (GHWF), *Trialeurodes vaporariorum* (Hoddle et al., 1997a,b). Since 1926, this tiny parasitic wasp has been used successfully in the United States and other countries through-

out the world to control this whitefly pest in greenhouses. However, although the existence of *E. formosa* has been known for almost 80 years, little information is available concerning the regulation of its development or that of its host. A uniparental parasitoid, *E. formosa* is free-living as an adult and passes its immature stages within the hemocoel of its host. Therefore, the successful growth

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and development of the parasitoid depends in great part upon the suitability of the host, which, in turn, varies with host age, size, and physiological condition (Vinson, 1988, 1990). Following successful oviposition, the ovipositing parasitoid and its larva manipulate the physiology of the host to create conditions that are suitable for the progeny to grow and mature. Parasitoids use a variety of methods to alter host biochemistry and physiology including the injection of virus, venom, or other regulatory factors at the time of oviposition (reviewed by Lawrence and Lanzrein, 1993; Lavine and Beckage, 1995; Strand and Pech, 1995; Beckage, 1997) as well as the release of regulatory compounds by the developing parasitoid (Brown et al., 1993; Schepers et al., 1998; Gelman et al., 1998). Since there has been considerable interest in the development of an in vitro system for rearing the endoparasitoid both for the short and long term, a study of the multitude of host-parasitoid interactions that occur upon parasitization and during the maturation of the parasitoid is important.

Nechols and Tauber (1977a) reported that the instar during which host parasitization occurred significantly influenced the progress of *E. formosa* development. However, the effect of host age (in this study, defined as host instar parasitized) on the growth of the parasitoid, i.e., body size of both nymph and adult, as well as on adult emergence pattern and longevity have not been studied. Also of interest is the effect of host age on the developmental progress of the parasitoid. Are host cues or triggers necessary for the parasitoid to molt or undergo metamorphosis? In this study, we describe the influence of the greenhouse whitefly instar parasitized on the growth, adult emergence pattern, and longevity of *E. formosa*. We also present information concerning the requirement of host cues for the parasitoid's molt to the 3rd instar.

MATERIALS AND METHODS

Whitefly Culture

T. vaporariorum was maintained ($T = 26 \pm 4^\circ\text{C}$; r.h. approximated 55%; photo-periodic regimen =

L:D 16:8) on a variety of plants [cotton, eggplant, green bean, hibiscus (provided by Yoder Brothers, Alba, FL), poinsettia (provided by Paul Ecke Ranch, Encinitas, CA), sunflower, sweet potato, and tomato] in a greenhouse. Eight fluorescent cool white 30-W bulbs (G30-TB, GE) were installed to maintain a light intensity of 6,000 lux. With the exception of poinsettia and hibiscus (cuttings and plants, respectively, provided by commercial growers), plants were started from seed, which were germinated in small 3-inch pots or in wells of multiwell germination trays. As necessary, plants were transferred to successively larger pots and were watered every Monday, Wednesday, and Friday and fertilized once per week.

Parasitoid Culture

E. formosa (Beltsville strain, collected from greenhouses in Beltsville, MD) were maintained in an incubator at $26 \pm 2^\circ\text{C}$, r. h., approximately 55%, a photo-periodic regimen of L:D 16:8 and a light intensity of 600 lux. Rooted green bean leaf cuttings were exposed to GHWFs for 20–24 h by placing the cuttings in a white poly-organza (48 mesh) bag containing ≥ 200 whiteflies. Infested cuttings were also maintained in an incubator at $26 \pm 2^\circ\text{C}$, r. h., approximately 55%, a photoperiodic regimen of L:D 16:8 and a light intensity of 600 lux. When most whitefly nymphs had reached the 3rd instar, cuttings were placed in 20-ml borosilicate glass vials (with screw caps in which a small hole had been drilled) that had been filled with water containing 0.01% plant food (Miracle-Go Products, Inc., Port Washington, NY). Vials were placed individually in clear plastic cylindrical cages (10×7 cm) containing approximately 100 *E. formosa* adults. After 4 h, parasitoids were removed and each cutting was transferred to an aquatube (no. 54, Syndicate Sale, Inc., Kokomo, IN) containing 0.01% plant food solution. Aquatubes were transferred to plastic Petri dishes (150×25 mm) that were placed in an incubator (environmental conditions as described above). To provide for air circulation, Petri dish lids were fashioned with an opening (11 cm in diameter) that had been covered with white poly-organza.

Cuttings were watered every Monday, Wednesday, and Friday. Upon emergence, parasitoid adults were transferred to plastic cylindrical cages and fed with drops of pure honey.

Parasitization

Green bean leaf cuttings were infested with GHWFs as described above. When the GHWFs had reached the appropriate stage [sessile first instar (0.5–1 day post-hatch), early 2nd, early 3rd or early 4th instar], they were exposed to adult *E. formosa*. GHWF nymphal instar was identified by measuring the body length and width of the whitefly and young instars were selected based on their relatively flat appearance (Nechols and Tauber, 1977b; Gelman et al., 2002). Parasitization (oviposition) was observed under a stereoscopic microscope, and with the aid of a fine-tip Gel-Writer pint pen (Paper-Mate, Japan), parasitized whitefly nymphs were marked immediately by placing a small dot next to each parasitized whitefly. Multi-parasitizations of a given nymph were not permitted. Parasitized whiteflies were returned to the incubator.

Determination of Rates of Parasitoid Growth and Development

To determine the effect of instar parasitized on the rate of *E. formosa* development, each day post-parasitization (until adult emergence) at least 10 parasitized whitefly nymphs/experiment were dissected and each experiment was repeated at least 3 times. A parasitized nymph was placed in a drop of saline and fine insect pins were used to remove the parasitoid from the nymph. In order to avoid bias, for each whitefly instar, the time between

parasitization and dissection was kept constant. The stage of the developing parasitoid was determined and recorded and the developmental time was noted. Host instar and the developmental stage of 4th instar hosts were also recorded. Fourth instar GHWFs were staged based on increasing body depth (Stages 1–5) and the development of the adult eye (Stages 6–9) (Gelman et al., 2002). The length of the parasitoid was measured using a calibrated ocular micrometer. Larval shape and body length of the various parasitoid larval instars were similar to those reported for *Encarsia pergandiella* by Gerling (1966) and for *E. formosa* by Agekyan (1982). A standard system for the identification of young parasitoid stages was established and is presented in Table 1. When adult parasitoids emerged, they were transferred to 40-ml clear plastic bottles and maintained in the incubator under the conditions described above. Adults were not fed and their longevity was recorded. Upon death, head width was determined using the calibrated ocular micrometer.

Data Analysis

At least three replications were performed for each of the four host instars parasitized. Data was analyzed using the statistics program STATISTIX (Analytical Software, Inc., Tallahassee, FL). To determine if there were significant differences in growth and development rates based on host instar parasitized, a one-way ANOVA followed by the Tukey HSD post hoc test was used.

Histological Methods

Whitefly nymphs parasitized as 3rd instars were fixed for 2–3 h in Carnoy's Formula 2; 60% abso-

TABLE 1. Characteristics for the Identification of Larval Stages of *Encarsia formosa*

Instar	Body size (L × W) (mm) ^a	Description of body shape
Young 1st	0.15 × 0.04–0.25 × 0.08	Cylindrical with a long tail (0.13 mm)
Old 1st	0.26 × 0.1–0.33 × 0.12	Cylindrical with a short tail (≈0.10 mm)
Young 2nd	0.35 × 0.13–0.52 × 0.15	Comma-shaped; tail has disappeared
Old 2nd	0.53 × 0.20–0.54 × 0.20	Comma-shaped
Young 3rd	0.55 × 0.20–0.75 × 0.30	Comma-shaped; head more pointed than in 2nd instar
Old 3rd	0.78 × 0.35–0.85 × 0.38	Similar to young 3rd, but with a prominent yellow gut

^aWidth measurement was determined at widest part of larva.

lute ethanol; 30% chloroform; 10% glacial acetic acid (Davenport, 1960). The fixed nymphs were rinsed with absolute ethanol, stained with 1% eosin b in absolute ethanol for 30 min, then washed with absolute ethanol to remove free eosin; this step stains the nymphs pink, allowing them to be more easily manipulated during embedding. The dehydrated nymphs were transferred through 4 changes of xylene and then placed in paraffin (Paraplast Xtra) at 60°C where they remained overnight. The whiteflies were then transferred to fresh paraffin in embedding molds, and chilled rapidly in ice water.

The embedded nymphs were sectioned at 5 µm on a rotary microtome. Sections were relaxed on water at 40°C, mounted on egg albumin-coated slides, dried, and placed horizontally in a drying oven at 40°C overnight.

Mounted sections were deparaffinized in 3 changes of xylene, transferred through 2 changes of absolute ethanol, and rehydrated through a series of aqueous ethanol solutions (95, 90, 70, and 50%). Sections were stained with Weigert's iron hematoxylin followed by Casson's trichrome as described by Kiernan (1990)

RESULTS

Identification of Parasitoid Instars

Photographs of 1st–3rd parasitoid instars are presented in Figure 1. Cross sections of a freshly deposited *E. formosa* egg, a young 1st, young 2nd, and older 3rd instar, fixed and embedded on days 0, 3, 5, and 7 post-oviposition, respectively, are pictured in Figure 2A–D, and provide more detail. Observations of body shape and dimensions made on dissected larvae were generally similar to body shape and length and width determinations observed for the parasitoid in histological sections. The tail of 1st instar larvae was clearly visible for both dissected and sectioned larvae with the anterior of 2nd and 3rd instars somewhat wider than the posterior, resulting in the comma-shaped appearance of these instars. Rapid development of the parasitoid nervous system and gut occurred be-

tween the 1st and 2nd instar, and in the 3rd instar conspicuous fat body development was observed. Third instar larvae also displayed well-developed musculature of the mouthparts, relative to earlier stages, and early ovarian development (not visible in Fig. 2D) was apparent in the last instar wasp.

Developmental Rates of *E. formosa*

E. formosa parasitized all four instars of the GHWF and was able to complete development no matter which instar was parasitized (Table 2). However, the developmental rates of the parasitoid differed significantly depending upon the host instar selected for parasitization. The wasp developed significantly faster when 3rd and 4th instar GHWFs were parasitized than when 1st and 2nd instars were parasitized (Table 2). The duration of parasitoid development from oviposition to adult emergence differed significantly for the four host instars ($F = 247.23$; $df = 3, 821$; $P = 0.0016$). When 1st instar whiteflies were parasitized, the duration was significantly longer than when 2nd–4th instars were parasitized. Similarly, parasitoid developmental time was significantly longer when 2nd instars were parasitized as compared to when 3rd or 4th instars were parasitized (Table 2). There were also important differences when durations of individual parasitoid stages were examined. The length of embryonic (time from oviposition to hatch) ($F = 23.95$; $df = 3, 110$; $P = 0.0000$) and 1st instar larval development ($F = 9.26$; $df = 3, 114$; $P = 0.0000$) varied depending upon the stage of the host at the time of parasitization. The duration of embryonic and 1st instar parasitoid development was significantly longer when 1st or 2nd instar hosts were parasitized than when 3rd or 4th instars served as hosts (Table 2). Developmental times for 2nd ($F = 3.19$, $df = 3, 224$; $P = 0.0000$) and 3rd instar ($F = 3.43$, $df = 3, 146$, $P = 0.0000$) parasitoids also differed significantly depending upon host age. Developmental time for both 2nd and 3rd instar parasitoids was significantly longer when 1st instar GHWFs were parasitized than when older GHWFs were parasitized (Table 2). Significant differences in the length of pupal development were

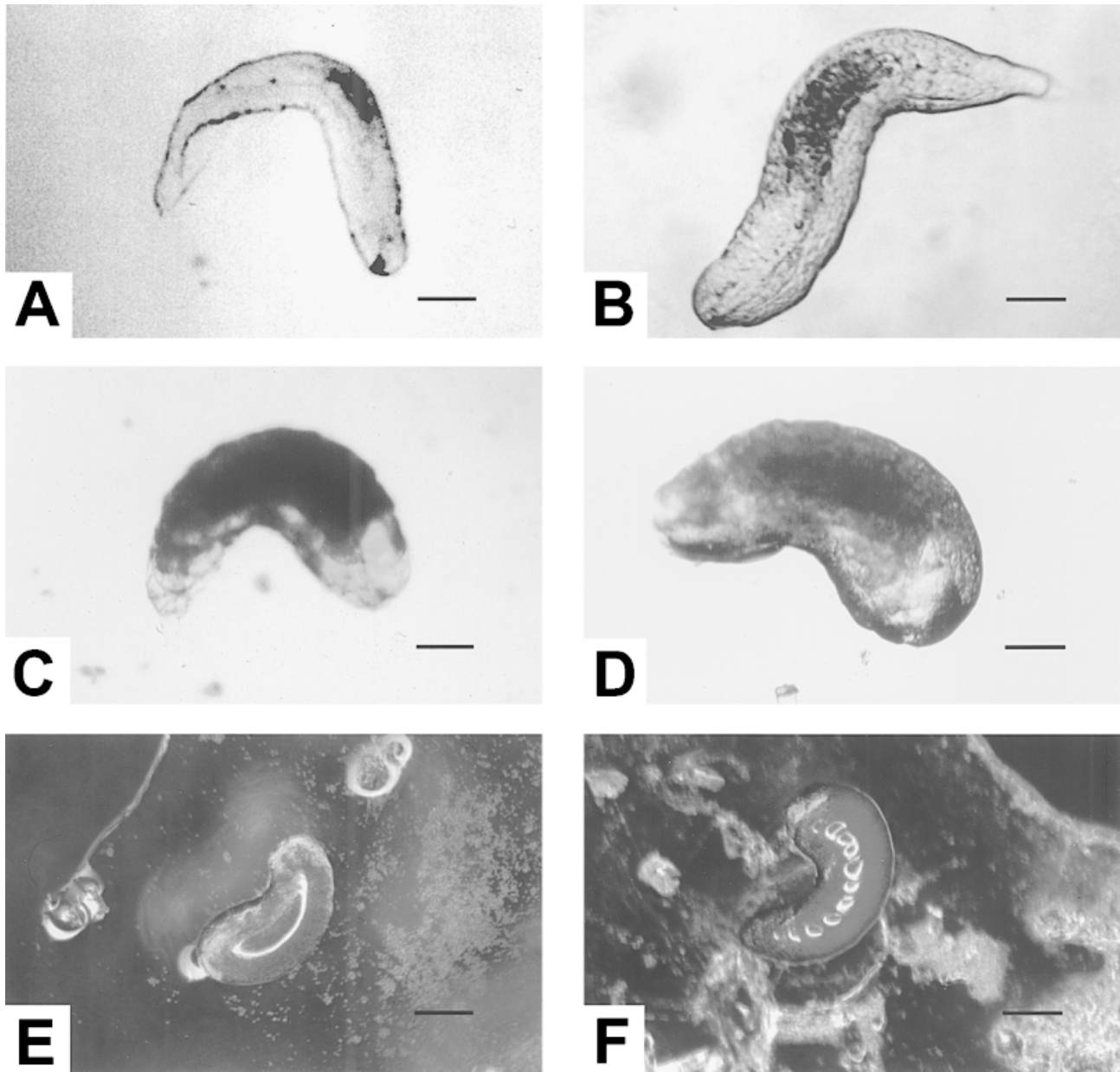


Fig. 1. Larvae of *E. formosa* as seen under the stereomicroscope. Parasitized whiteflies were placed individually in drops of saline and parasitoids were carefully removed with the aid of fine insect pins. A = an early 1st instar, B = a late 1st instar, C = an early 2nd instar, D = a

late 2nd instar, E = an early 3rd instar, F = a late 3rd instar. Bars (A–E) = 0.0385, 0.0438, 0.0900, 0.0964, 0.1719, and 0.1750 mm, respectively. See Table 1 for descriptive details.

also observed ($F = 18.41$, $df = 3, 429$; $P = 0.0000$) When parasitization occurred in 1st instar GHWFs, parasitoid pupal development took significantly longer than when later instars were selected as hosts, whereas parasitization during the 3rd instar, as compared to all other instars, resulted in sig-

nificantly shorter pupal development time (Table 2). It should be noted that with the exception of the 1st instar parasitoid, mean developmental times of each parasitoid stage were shorter (although not always significantly so) when oviposition occurred in 3rd instar GHWFs.

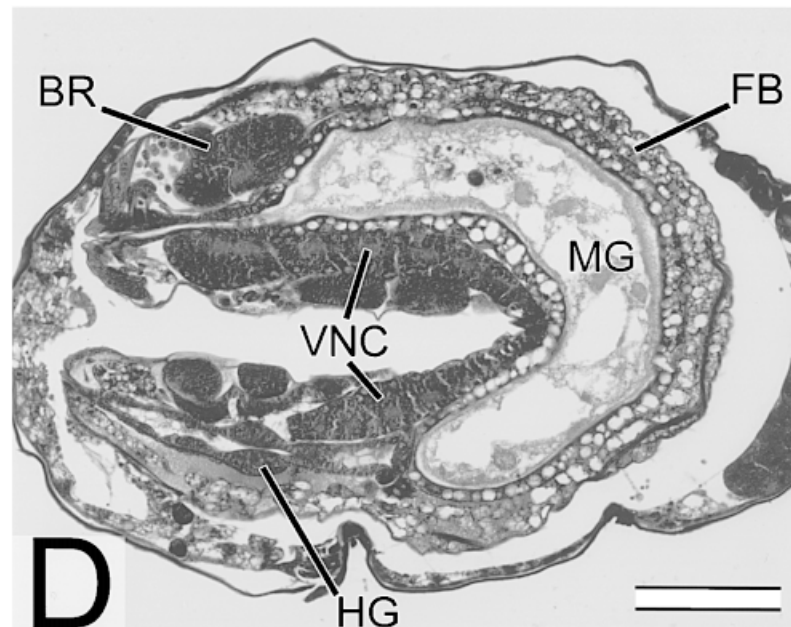
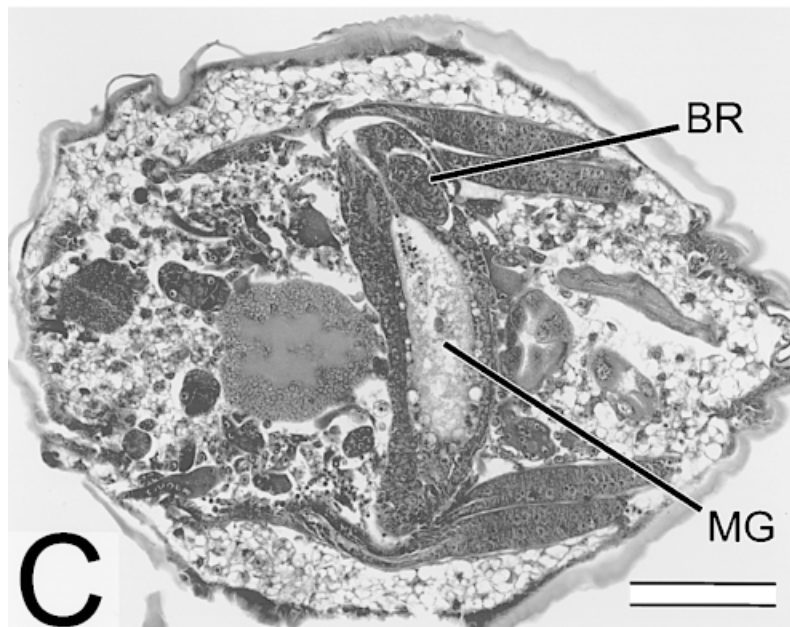
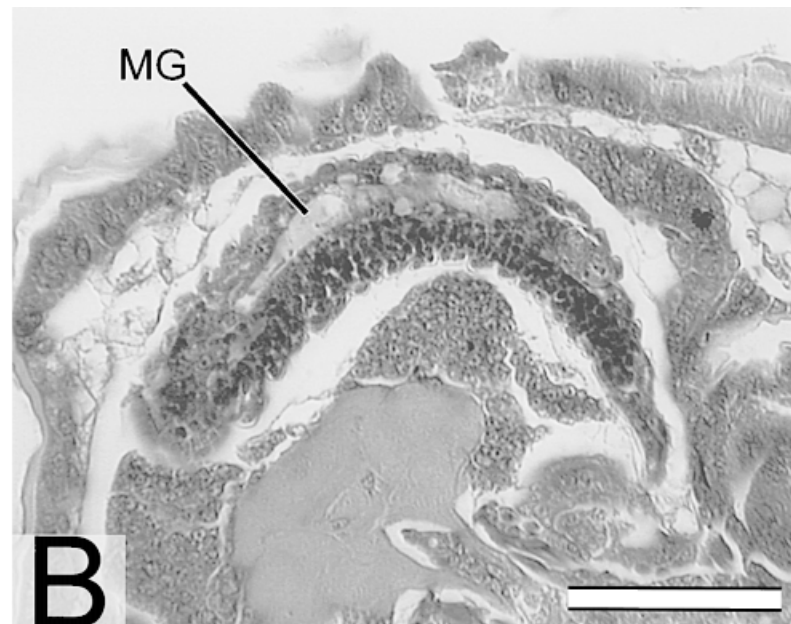


Fig. 2.

TABLE 2. Effect of Greenhouse Whitefly Instar Parasitized on the Developmental Duration of *Encarsia formosa**

Host instar parasitized	No. of hosts parasitized	Developmental duration of the parasitoid (day \pm SD)					
		Embryo	1st instar	2nd instar	3rd instar	Pupa	Egg-adult
1st	314	7.28 \pm 2.77 ^a	3.02 \pm 0.54 ^a	2.85 \pm 0.13 ^a	3.58 \pm 1.09 ^a	4.65 \pm 1.45 ^a	21.15 \pm 1.69 ^a
2nd	192	6.34 \pm 1.97 ^a	2.67 \pm 0.64 ^a	1.80 \pm 0.65 ^b	3.17 \pm 0.08 ^b	3.97 \pm 1.86 ^b	18.43 \pm 1.86 ^b
3rd	363	4.11 \pm 1.22 ^b	1.35 \pm 0.12 ^b	1.69 \pm 0.21 ^b	3.04 \pm 0.41 ^b	3.48 \pm 0.15 ^c	14.21 \pm 1.32 ^c
4th	294	4.18 \pm 0.97 ^b	1.33 \pm 0.07 ^b	1.88 \pm 0.19 ^b	3.10 \pm 0.02 ^b	4.01 \pm 0.11 ^b	14.42 \pm 1.62 ^c

*Duration for each parasitoid instar and the pupa was determined by subtracting the mean day of a given stage from the mean day of the following stage. Each value represents the mean \pm S.D. of at least 3 separate determinations. A one-way ANOVA followed by the Tukey's HSD post hoc test were used to determine if there were significant differences in developmental duration. Means in the same column followed by a different letter are significantly different.

Emergence Rate of *E. formosa* and Adult Longevity

Host age did not have an effect on percent emergence of *E. formosa*, which ranged between 95 and 99% (Table 3). However, there were significant differences in adult parasitoid longevity ($F = 41.64$; $df = 3,821$; $P = 0.0000$). Adult wasps lived significantly longer when they emerged from GHWFs parasitized as 3rd and 4th instars than from hosts parasitized as 1st and 2nd instars (Table 3).

Emergence Pattern of *E. formosa*

The adult wasp emergence pattern varied greatly depending upon the whitefly instar selected for parasitization (Fig. 3). When 3rd and 4th instar GHWFs were parasitized, emergence peaked on the 2nd day after emergence was first observed (60 and 50% wasp emergence for 3rd and 4th instar hosts, respectively), and the total emergence period was 5 days. The number of *E. formosa* emerging from hosts parasitized during the 2nd instar also peaked on the 2nd day of emergence, but the peak was significantly lower and the duration of the emergence period increased to 8 days. In the case of parasitoids emerging from hosts parasitized as 1st instar nymphs, peak emergence occurred on day 5 (approximately 20% emergence), and emergence continued for 11 days.

Fig. 2. Cross sections of whiteflies parasitized by *E. formosa*. Sections were prepared as described in Materials and Methods. A = a parasitoid egg (location is indicated by pointer); B = a young 1st instar; C = a young 2nd instar; D = an older 3rd instar. BR = brain; FB = fat body; HG = hindgut; MG = midgut; VNC = ventral nerve cord. Bars (A,B) = 50 μ m, (C,D) = 100 μ m.

Size of *E. formosa*

Larval body length and adult head width of *E. formosa* were measured to determine the effect of host age on parasitoid growth (Table 4). In general, the time of parasitization did not have a significant effect on the size that the parasitoid attained at each stage measured. However, parasitoids were slightly larger when they developed only in 3rd and 4th instar whiteflies and 2nd instar *E. formosa* were significantly larger when parasitization occurred in the 4th instar than when parasitization occurred in earlier instars (Table 4).

Developmental Chronology of *E. formosa*

Depending upon the whitefly instar parasitized, the presence of a given parasitoid instar in a particular host instar varied (Fig. 4). The younger the instar parasitized, the less synchronous was parasitoid development, and the parasitoid was detected in more host instars. Thus, when 1st instar GHWFs were selected for para-

TABLE 3. Effect of Greenhouse Whitefly Instar Parasitized on Emergence Rate and Longevity of *Encarsia formosa* Adults*

Host instar	No. of hosts parasitized	Emergence rate (%) (\pm SD)	Longevity (day) (\pm SD)
1st	153	95.05 \pm 5.73 ^a	1.99 \pm 0.59 ^a
2nd	182	97.65 \pm 2.35 ^a	2.06 \pm 0.36 ^a
3rd	224	96.12 \pm 3.35 ^a	2.57 \pm 0.61 ^b
4th	266	98.80 \pm 1.70 ^a	2.54 \pm 0.58 ^b

*For emergence rate and longevity, respectively, each value represents the mean \pm S.D. of at least 3 and 150 separate determinations. A one-way ANOVA followed by the Tukey's HSD post hoc test was used to determine if there were significant differences in adult emergence rate and longevity of *E. formosa* based on the host instar parasitized. Days of parasitoid adult survival served as a measure of adult longevity. Means in the same column followed by a different letter are significantly different.

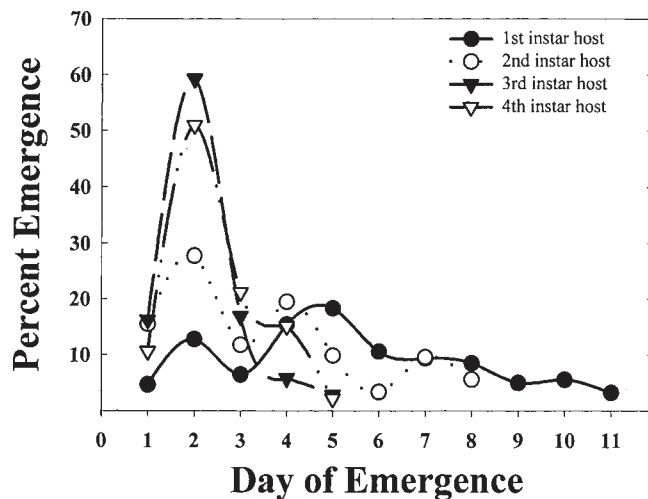


Fig. 3. Emergence pattern of *E. formosa* as a function of host instar parasitized. Parasitized whiteflies were examined daily and the time of parasitoid emergence was recorded. Each point equals the mean of at least three separate determinations. To avoid confusion, standard errors have not been indicated. However, for any given point, the value of the standard error was $\leq 10\%$ of the point's value.

sitization (Fig. 4A), the 1st instar parasitoid larva was observed in 2nd, 3rd, and 4th instar whiteflies and the 2nd instar parasitoid was found in 3rd and 4th instar hosts. When 2nd instar whiteflies were selected (Fig. 4B), 1st instar parasitoids were also present in 2nd–4th instar hosts, but the 2nd instar parasitoid was not observed until the whitefly had molted to the 4th instar. When 3rd instar whiteflies were parasitized (Fig. 4C), both 1st and 2nd instar parasitoid larvae were found only in 4th instar hosts. Importantly, however, no matter which instar was parasitized, the parasitoid never molted to its last instar until the host had reached Stage 5 of its last instar (Fig. 4).

DISCUSSION

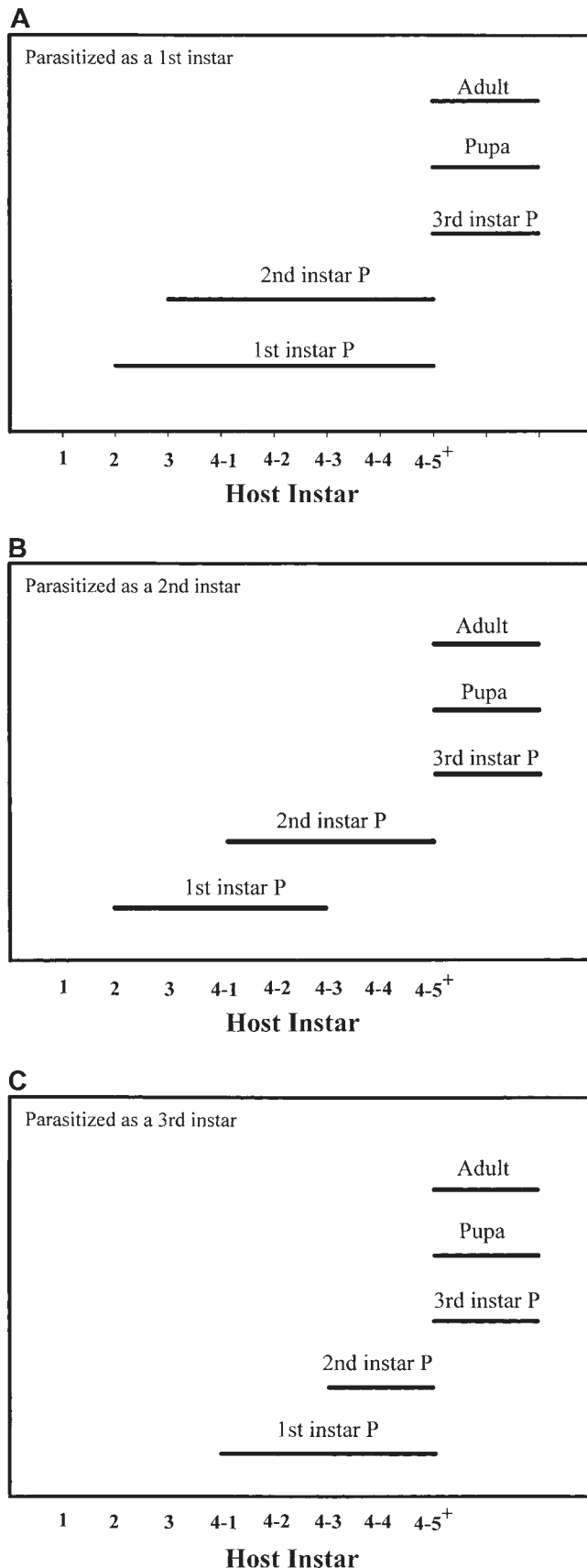
When only a single host instar was available for parasitization, *E. formosa* females were equally active in attacking all four instars of the GHWF and parasitoid offspring successfully completed their life cycle. However, *E. formosa* developed significantly faster when 3rd or 4th instar GHWFs were parasitized than when 1st or 2nd instars were parasitized. These results agree with those reported by Nechols and Tauber (1977a). As reviewed by Vet et al. (1980), when selecting a host for parasitization, *E. formosa* actually show a preference for 3rd and early 4th instar GHWFs. In our investigations, parasitoid development occurred at its maximal rate when 3rd instar GHWFs were parasitized. The rapid growth of the parasitoid larvae was especially evident when viewed in cross section. Although the mean duration of the 1st instar was only 1.35 days, the development of the nervous system and gut was far more advanced in 2nd instar than in 1st instar larvae.

In contrast to results reported by Nechols and Tauber (1977a), i.e., no effect of instar parasitized on the length of the pupal stage of the parasitoid, we report that the duration of the pupal stage of *E. formosa* was significantly different depending upon the host age at the time of parasitization. In our investigations, there were three groups in which the mean pupal parasitoid durations were significantly different from each other, parasitization of 1st, 2nd/4th, and 3rd instar whiteflies. For these three groups, the relative durations of the pupal stage of the parasitoid were long (4.7 days), medium (4.0 days), and short (3.5 days), respectively, as compared to Nechols and Tauber's report of ap-

TABLE 4. Effect of Greenhouse Whitefly Instar Parasitized on the Growth of *Encarsia formosa*

Host instar	No. of hosts parasitized	Mean length of the parasitoid (mm \pm SD)				
		1st instar	2nd instar	3rd instar	Pupa	Adult head width
1st	275	0.24 \pm 0.05 ^a	0.39 \pm 0.01 ^a	0.65 \pm 0.04 ^a	0.65 \pm 0.04 ^a	0.26 \pm 0.01 ^a
2nd	318	0.25 \pm 0.06 ^a	0.40 \pm 0.04 ^a	0.66 \pm 0.04 ^a	0.66 \pm 0.05 ^a	0.27 \pm 0.02 ^a
3rd	377	0.26 \pm 0.04 ^a	0.41 \pm 0.03 ^a	0.70 \pm 0.07 ^a	0.68 \pm 0.05 ^a	0.27 \pm 0.02 ^a
4th	422	0.29 \pm 0.04 ^a	0.55 \pm 0.13 ^b	0.72 \pm 0.10 ^a	0.70 \pm 0.03 ^a	0.27 \pm 0.02 ^a

Mean length and mean adult head width were used as growth parameters for the parasitoid. Each value represents the mean \pm S.D. of at least 100 separate determinations. A one-way ANOVA followed by the Tukey HSD post hoc test was used to determine if there were significant differences in body sizes of *E. formosa* based on the greenhouse whitefly instar parasitized. Means in the same column followed by a same letter are not significantly different.



proximately 7.5 days for all groups when host *T. vaporariorum* were reared on tobacco. Interestingly, the effect of host age on the total time of parasitoid development (egg through adult stage) was relatively similar in the two studies. However, since Nechols and Tauber did not provide duration times based on host age for all instars of *E. formosa*, we were unable to make comparisons for individual parasitoid instars.

Host age/size significantly influenced adult longevity as well as developmental rates of *E. formosa* (Tables 2 and 3). Since *E. formosa* is an endoparasitoid, its larva lives within the hemocoel of its host, and relies upon the nutrients present in its host's hemolymph for growth and survival. Therefore, the quality as well as the quantity of hemolymph would be expected to critically affect parasitoid maturation as well as adult viability. Since younger, smaller hosts (1st and 2nd instars) have less hemolymph, the supply of nutrients may be insufficient to satisfy the requirements of the developing parasitoid. In addition, if *E. formosa* induces the synthesis of "parasitism-specific" proteins in its host [as has been reported in other host parasite systems (reviewed in Beckage, 1993)], these proteins may not be as readily synthesized in younger as in older hosts. It is also possible that the parasitoid may simply sense that its host's size is too small to support the development of older parasitoid instars and, therefore, slows its own maturation rate. Whatever the cause, slower growth and development of parasitoid larvae as well as a

Fig. 4. Developmental chronology of *E. formosa* as a function of host instar parasitized. A–C: Whiteflies parasitized as early 1st, 2nd, and 3rd instars, respectively. At least 10 parasitized whitefly nymphs were dissected each day post-parasitization and the stage of parasitoid development was ascertained. The whitefly instar, and for the 4th instar, stage (x-axis) in which each parasitoid instar, pupa, or adult was present is represented by a horizontal line. For example, when a first instar whitefly was parasitized, 1st instar parasitoids were detected in 2nd, 3rd, and 4th instar hosts, and 2nd instar parasitoids were detected in 3rd and 4th (Stages 1–5) instar hosts. P = parasitoid; Pupa = parasitoid pupa; Adult = adult wasp.

shorter life span of adult parasitoids were observed when younger, smaller 1st and 2nd instar hosts were parasitized as compared to when development was entirely in older 3rd and 4th instar hosts.

Effect of host size/age also affected the emergence pattern of *E. formosa*. When the parasitoid began its development in younger hosts, the emergence pattern was flatter than when development was only in older hosts. Since parasitization of 1st and 2nd instar hosts effects longer parasitoid developmental times that, in turn, tend to be associated with increased asynchrony, it is not surprising that emergence was prolonged when younger instars were used for parasitization. Nevertheless, *E. formosa* was able to complete its life cycle regardless of which host instar was parasitized and was thus able to overcome the suboptimal conditions provided by 1st and 2nd instar hosts. However, it was unexpected that the growth (as determined by body size) and percent adult emergence of *E. formosa* were not influenced by host age, since, as mentioned previously, reduced adult longevity was observed when parasitoids began their development in 1st and/or 2nd instars of the GHWF.

It is well documented that in many host-parasite systems, host age at the time of parasitization affects parasitoid growth and development (Smilowitz and Iwantsch, 1973; Beckage and Riddiford, 1978; Pennacchio et al., 1993; Harvey et al., 1999; Hu and Vinson, 2000). Thus, when *H. virescens* is parasitized by *Cardiochiles nigriceps* (Hymenoptera: Braconidae), the duration from oviposition to adult emergence is significantly longer when younger hosts are parasitized than when older hosts are parasitized (Pennacchio et al., 1993), primarily because the parasitoid will not molt to its 2nd instar until the host has molted to its last instar. Similarly for *Manduca sexta* (Lepidoptera, Noctuidae) parasitized by *Cotesia congregata* (Hymenoptera, Braconidae), parasitization of larger hosts favors more rapid development of the parasitoid (Beckage and Riddiford, 1978). In contrast, the developmental time of *Campolitis sonorensis* (Hymenoptera: Ichneumonidae) from egg to adult emergence is significantly less when its host, *Heliothis virescens* (Lepidoptera, Noctuidae), is parasitized as a 1st in-

star than when parasitization occurs in 4th instars (Hu and Vinson, 2000). Development of younger stages (embryonic and 1st instar) is faster in younger hosts than in older hosts, i.e., there is a stage specific pattern. For *Pieris rapae* and *Pieris brassicae* (Lepidoptera, Pieridae) parasitized by *Cotesia rubecula* (Hymenoptera: Braconidae), parasitoids also completed development faster, and in addition, generally survived better and grew larger in earlier than in later host instars (Harvey et al., 1999). Thus, depending upon the host-parasite system under investigation, the effect of host age on parasitoid development varies, even within the same genus. The developmental pattern of *E. formosa* more closely resembles that of *C. nigriceps* and *C. congregata* than that of *C. sonorensis* or *C. rubecula*, i.e., when 1st and 2nd instars of the GHWF were parasitized, *E. formosa* developed more slowly than when older instars were parasitized. In addition to tracking the duration of parasitoid instars as a function of host age, we also determined if a given host instar was permissive in regard to the development of a specific parasitoid instar. When 1st and 3rd instar GHWFs were parasitized, *E. formosa* eggs hatched in successive host instars, but when 2nd instar GHWFs were parasitized, hatch was observed to occur in the same as well as in the next instar. Parasitoid ecdysis to the 2nd instar occurred in either 3rd or 4th instar hosts. Therefore, for hatch and for the 1st to 2nd instar molt, there does not appear to be a given instar that is permissive for these events. These results are in contrast to those of Nechols and Tauber (1977a) who reported that parasitoids do not molt to the 2nd instar until hosts have reached the 4th instar. The reason(s) for the discrepancy are unknown; perhaps the identity of the host plant, the strain of *E. formosa* used, or differences in environmental rearing conditions or staging techniques are responsible. Importantly, in our investigations, the parasitoid never molted to its last instar until the host had reached Stage-5 of its last instar, the stage in which pharate adult development has been initiated (Gelman et al., 2002). Thus, a condition(s) associated with host pharate adult development appears to be required for the parasitoid's final lar-

val molt. Permissiveness associated with the biochemical/physiological milieu of a particular host instar or stage is not unique to host-parasite interactions between *E. formosa* and *T. vaporariorum*. As mentioned previously, in the *C. nigriceps*-*H. virescens* system, when 1st instar hosts are parasitized, the egg hatches, but the 1st instar parasitoid does not molt to the 2nd instar until the host has reached its last (5th) instar (Pennacchio et al., 1993); and, in the *Manduca sexta*-*Cotesia congregata* system, no matter which instar is parasitized, the parasitoid does not molt to its 2nd instar until the host has reached its last instar (Beckage and Riddiford, 1983). These differences in parasitoid physiologically-based behavior may be related to the different nutritional requirements and/or hemolymph nutrient utilization strategies that, in turn, have become evolutionarily linked to hormonal cues associated with particular stages of host development.

In summary, *E. formosa* exhibited an improved developmental rate, synchrony of adult emergence and adult longevity, when 3rd and 4th instar whiteflies were parasitized than when younger instars were parasitized. In contrast to previous reports, *E. formosa* 2nd instars were observed in 3rd instar host whiteflies. However, the parasitoid was never observed to molt to its last instar until its whitefly host had reached Stage 5 of the 4th instar, a stage associated with the early stages of *T. vaporariorum* pharate adult development. Therefore, it appears that one or more host cues are required for the final, rather than the 1st larval molt of *E. formosa*.

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